Developmental Biology 344 (2010) 487–496

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Gene Regulation

Program/Abstract # 251

1 + 1 = 3: When two hormones are better than one

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Auxin and brassinosteroids are two plant hormones that work synergistically and interdependently to promote growth and proper development in many plant species. While several co-regulated early response genes have been identified and there are well-characterized transcription factors from each pathway, it remains an open question how the auxin and brassinosteroid signals are integrated at the transcriptional level. We are using the auxin- and brassinosteroid-responsive gene SAUR-15 as a tool to dissect this problem. Using transgenic plants expressing reporter genes under the control of different portions of the upstream regulatory region of SAUR-15, we are working to determine the minimal region that retains response to each hormone separately and to both together. We are also disrupting known cis-regulatory elements to identify critical elements. We are also taking a comparative approach using previous work on auxin-responsive promoters in various plant species including soybean and pea. Together, these results will help us determine the molecular mechanism for how auxin and brassinosteroids work synergistically to induce expression of shared genes as well as increase our understanding about hormone interactions and how the regulation of hormones modifies plant development.

doi:10.1016/j.ydbio.2010.05.414

Program/Abstract # 252

Dll-B knockdown and overexpression in the ascidian *Ciona intestinalis*

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The Dll-B homeobox transcription factor in the simple chordate *Ciona intestinalis* is expressed in the entire animal hemisphere, fated to produce the ectoderm, in blastula and gastrula stages. We used transgenic knockdown and overexpression strategies to perturb normal Ci-Dll-B expression. We are further investigating the effects of this misexpression through quantitative RT-PCR analysis of putative Ci-Dll-B downstream targets and use of molecular markers to examine phenotype.

doi:10.1016/j.ydbio.2010.05.415

Program/Abstract # 253

Targeted mutagenesis in the sea urchin embryo using zinc-finger nucleases

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Gene targeting is one of the most powerful approaches to get insight into the function of specific genes during animal development. However, this approach is available only in a few models. Recently, targeted mutagenesis using engineered zinc-finger nucleases (ZFNs) has been reported in several model organisms. ZFNs consist of a zinc-finger DNA binding array and a nuclease domain of the restriction enzyme FokI and facilitate to introduce mutations at a specific genomic locus. In this study, we applied ZFN technology to the sea urchin, *Hemicentrotus pulcherrimus*. Efficient screening of functional ZFNs was achieved by a combinatorial use of a bacterial one-hybrid system using zinc-finger randomized libraries and a single-strand annealing assay. To evaluate the availability of ZFNs in sea urchin, we selected a pair of ZFNs for *HpHesC*. By injection of the *HpHesC* ZFN mRNAs, 10% of embryos showed a phenotype in which primary mesenchyme cell population increases as embryos injected with antisense morpholino oligonucleotides against *HpHesC*. In addition, sequence analysis of mutations showed that deletion (2%) and insertion (23%) occurred at the *HpHesC* target site in the embryos injected with *HpHesC* ZFN mRNAs. These results indicate that engineered ZFNs can introduce mutations into a specific genomic site in the sea urchin embryos. ZFN technology is also expected to be available to homology-directed gene insertion at target locus in several model organisms.

doi:10.1016/j.ydbio.2010.05.416

Program/Abstract # 254

Expression of MMP14 and MMP17 during sea urchin development

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Matrix metalloproteases (MMPs) are involved in many normal and pathological processes, including extracellular matrix fibrolysis, angiogenesis, and tumor cell metastasis. MMP structure is highly conserved among many species. We have searched the annotation of the sea urchin genome and found a number of previously uncharacterized MMP genes. We selected several of the more than twenty MMPs present in the genome for further analysis. Our current study is